SOME EFFECTS OF TEMPERATURE ON THE FREQUENCY OF DIVISION AND ON THE VOLUME OF STARCH AND FAT OF *CHILOMONAS PARAMECIUM*

A DISSERTATION

SUBMITTED TO THE BOARD OF UNIVERSITY STUDIES
IN CONFORMITY WITH THE REQUIREMENT FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

1939

JAY A. SMITH

Baltimore

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SOME EFFECTS OF TEMPERATURE ON THE FREQUENCY OF DIVISION AND ON THE VOLUME OF STARCH AND FAT IN CHILOMONAS PARAMECIUM

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Introduction

The present experiments were undertaken to further the knowledge of the metabolism of *Chilomonas,* using temperature as the variable factor, and noting its effects on the frequency of division and on the volume of starch and fat.

Chilomonas paramecium is a small biflagellated protozoan of elliptical shape, filled with numerous round particles. It has been known since the publication of Ehrenberg (1838), in which it was described, pictured and named. Its particles were noted but incorrectly described as food vacuoles. Since then many investigators have contributed to the knowledge of this organism. Schneider (1854) recognized the starchy nature of some of the particles. Fisch (1885) and Dangeard (1910) described chilomonads and added briefly to the knowledge of the organism.

Mast and Pace (1932*a*, 1932*b*, 1933, 1935, 1937, 1938) made two important contributions to the study of *Chilomonas.* First, they developed methods for cultivating the species in a bacteria-free solution of inorganic salts plus sodium acetate; second, they discovered that some of the particles in the chilomonads were composed of fat. In their experiments they observed the frequency of division and the volume of starch and of fat in various solutions at a constant temperature.

Chilomonas possesses four properties that make it admirably suited for physiological experiments. First, using the methods of Mast and Pace, it can be grown in sterile solutions containing relatively simple solutes. Second, it divides at a fairly uniform rate. Third, it lives and

[1] The author gratefully acknowledges the valuable suggestions of Dr. S. O. Mast and Dr. D. M. Pace, the patience of Mrs. L. Carper in obtaining equipment and supplies, and the many tolerant criticisms of Dr. M. E. Smith and others in preparing the manuscript, figures, and tables.

thrives in a temperature range extending over twenty degrees. Fourth, it possesses a fairly uniform number of particles of starch and fat which are undoubtedly forms of stored food material.

Materials and Methods

The organisms were obtained from a strain grown in this laboratory for many years and cultivated exclusively in bacteria-free solutions containing sodium acetate and inorganic salts. In recent years, the strain had been kept at temperatures between 24° and 27° C.

Table I

Culture solutions for *Chilomonas paramecium*. The acetate ammonium solution is that of Mast and Pace (1938); the acetate-free solution is a modification free of nutritive substances.

	Acetate ammonium solution	Acetate-free solution
Sodium acetate, $NaC_2H_3O_2.3H_2O$	248.8 mg.	—
Ammonium chloride, NH_4Cl	46.0	46.0 mg.
Dipotassium hydrogen phosphate, K_2HPO_4	20.0	20.0
Ammonium sulphate, $(NH_4)_2SO_4$	10.0	10.0
Magnesium chloride, $MgCl_2$	1.0	1.0
Calcium chloride, $CaCl_2$	1.16	1.16
Sodium chloride, $NaCl$	—	112.5
Sodium sulphate, $Na_2SO_4 \cdot 7H_2O$	—	47.0
Distilled water	100.0 ml.	100.0 ml.

The culture solutions used in the experiments are described in Table I. The acetate ammonium solution is that of Mast and Pace (1938) and was used in all experiments requiring a nutrient medium; the acetate-free solution was used in experiments in which no food was supplied to the chilomonads.

The water used in making all solutions was redistilled in a Pyrex still and condenser and stored in Pyrex flasks until used.

The chemicals were Kahlbaum's " für Analyses " or " highest purity " except the sodium chloride which was Merck's " for biological purposes." The calcium and magnesium chlorides were weighed out approximately and dissolved in water, the molarity of the solution ascertained by the Mohr (1856) method for determination of chlorides, and the solutions

were then diluted to the required concentration. The required amounts of the other salts were weighed out and dissolved in the required volume of water.

Sterility of all solutions was maintained by heating in a hot air oven at 85° C. for 30 minutes on each of three days or by heating in an autoclave at 15 pounds pressure for 15 minutes. Cultures contaminated with bacteria or mold appeared occasionally but were discarded immediately and replaced by uncontaminated cultures. The results presented in this paper were obtained with chilomonads growing in sterile solutions.

All new slides and glass tubing were thoroughly cleaned by boiling in a strong aqueous solution of soap, rinsing, immersing in sulphuric-chromic acid cleaning fluid, and rinsing thoroughly in tap and distilled water. The culture slides were made of Pyrex glass and each had two depressions; these were placed in clean square Petri dishes, two slides to each dish, and the assembly sterilized in a hot air oven at 150° C. for 60 minutes.

Two types of pipettes were used, the "capillary pipettes" for isolating individual chilomonads, and the "measuring pipettes" for measuring and transferring solutions. Both types were made as follows: Pyrex tubing was cut into appropriate lengths, one end of each length was flanged to hold a medicine-dropper bulb and stoppered with cotton. The other end was drawn to a capillary point or calibrated to measure 0.1 cc. of fluid. Five such pipettes were put into test tubes, which were stoppered and sterilized at 150° C. for one hour. The pipettes were rinsed and sterilized after each usage.

Eight temperatures, each constant to $\pm 0.25°$ C., were available in a large rectangular bath. A compartment at one end contained a refrigerating unit, another at the other end a heating unit, and both were controlled by Aminco Metastatic thermoregulators. Stirrers provided a uniform temperature gradient between the two end compartments. Additional temperatures constant to $\pm 0.5°$ C. were available in a small bath.

In all experiments on the frequency of division the "isolation culture" method was employed. In this method, one chilomonad was isolated in a depression; the frequency of division was calculated from the number of progeny produced in a given time. Isolation cultures were prepared as follows. The hydrogen ion concentration of the acetate ammonium solution, prepared and sterilized as described above, was adjusted to pH 6.8 with sodium hydroxide or hydrochloric acid. A small quantity (0.1 cc.) was put into the depressions of clean, sterile slides in Petri dishes. A few cubic centimeters of water were poured into the bottom, and the dishes were put into the bath at constant temperature. After the temperature of the solution had reached that of the bath, one

chilomonad was introduced to each depression. The dish was left undisturbed for about 24 hours, and then the number of chilomonads in each depression ascertained. One of these chilomonads from each depression was transferred to the corresponding depression of a new dish and the process repeated as often as desired. The number of chilomonads in eight depressions was determined in each observation.

From the counts of chilomonads obtained in this manner, the frequency of division in divisions per hour was calculated as follows. The mean number of chilomonads per depression was computed. The number in the geometric progression of 2 immediately below the mean was subtracted from the mean; the remainder was divided by the same number in the geometric progression of 2 to give the fraction of divisions more than the last complete division. The result was added to the number of complete divisions. An example will elucidate the method. If 59 chilomonads were found in 8 depressions, then each had an average of 7.4 chilomonads. The number in the geometric progression of 2 immediately below 7.4 is 4, representing 2 complete divisions. Thus, 4 was subtracted from 7.4, and the difference, 3.4, divided by 4 to give 0.85 divisions more than the last complete division. The total number of divisions was 2 plus 0.85, or 2.85 divisions.[2] Convenient tables were constructed to abbreviate the calculations. The final value was last divided by the number of hours between observations to give divisions per hour. The method of computing the number of divisions is given here in detail because it differs radically from the method used by Mast and Pace (1933, 1935) for determining the frequency of division of chilomonads in isolation cultures.

There was much variation in the frequency of division and in the volume of starch and of fat under identical conditions of nutrition and temperature, so statistical methods were used to ascertain significant differences. The methods are those described in standard textbooks of statistics, such as Gavett (1925).

[2] It must be pointed out that the method described here is actually only a method of first approximation. The exact number of divisions can be determined from any number of organisms by applying the following equation:

$$X = \frac{\log N}{\log 2},$$

where N is the average number of chilomonads per depression, and X is the number of divisions.

Experimental Results

The Effect of Temperature on the Frequency of Division of Chilomonas paramecium

Chilomonads were grown exclusively in isolation cultures at all temperatures available, and the frequency of division measured. The results of this experiment are presented in Fig. 1. The figure shows that the frequency of division rises from zero at 9.5° C. to a maximum at 26°–30° C., and then decreases to zero at 35° C. Below 30° C. the measured frequency of division reflects correctly the rate of reproduction because the organisms live and thrive indefinitely. Above this temperature, however, the actual frequency of division may or may not

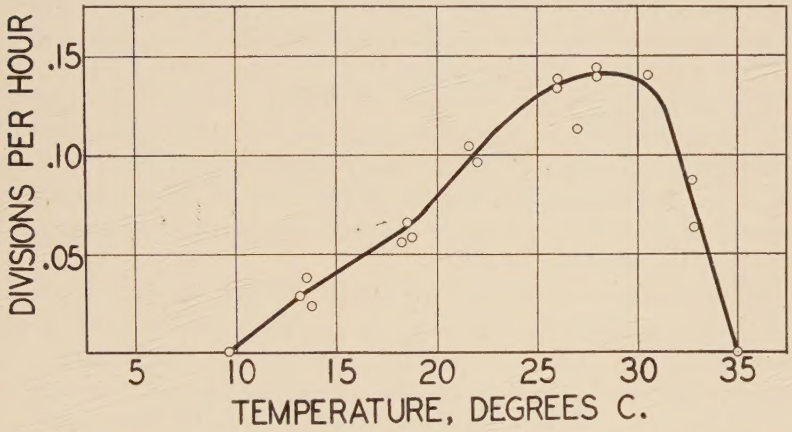

Fig. 1. The relation between temperature and the frequency of division of *Chilomonas paramecium.*

increase; the actual change is obscured by a decrease in the number of chilomonads brought about by death.

These experiments indicate that *Chilomonas,* like other living organisms, exhibits an increase in the metabolic activity (measured by the frequency of division) as the temperature rises to an optimum and then a decrease as the temperature rises still further. The reader is referred to Bělehrádek (1935) for an extensive review of this phenomenon.

An interesting observation was made in some preliminary experiments: for a short time after transfer of chilomonads from lower to higher temperatures, their frequency of division was low; gradually, however, it increased to that characteristic of the higher temperature. The experiment was repeated as follows to measure this phenomenon accurately and to seek other effects of low temperature on the frequency of division.

The organisms, apparatus, and materials were the same as in the preceding experiment. A clone derived from one chilomonad was grown at 27° C., and several from this clone were transferred to each of about 80 depressions and allowed to divide extensively at 27° C. These "stock cultures" were transferred to the compartment at 13.5° C. and left one week. Then isolation cultures were prepared and transferred to the six higher temperatures, where they were maintained and observed for two weeks. At the same time, the "stock cultures" were transferred to the compartment at 9.5° C. where they were kept for four weeks or until all chilomonads died. Isolation cultures were made from the "stock cultures" at intervals of one week, transferred to the seven higher temperatures, maintained and observed for a period of two weeks after establishment or, as was the case at 39° and 35° C., until all chilomonads died. The results are presented in detail in Figs. 2 and 3.

Figure 2 shows that during the first 48 hours after transfer from the low temperatures, the frequency of division increased from zero to that characteristic of the higher temperatures. Furthermore, after this period, the frequency of division remained constant at a value characteristic of the temperature studied, except that of those chilomonads exposed to 9.5° C. for 3 and 4 weeks, respectively, and observed at 30° C., which decreased to zero.

From this figure, three facts are evident. First, the exposure to low temperature modified the organisms so that their frequency of division was inhibited for a time after removal from the low temperature. Second, after the period of recovery from the effects of low temperature, the frequency of division became constant at a value characteristic of the temperature studied; in other words, exposure to low temperature had no permanent effect on the frequency of division. Third, all exposures to low temperature longer than one week had identical effects on the frequency of division at higher temperatures in which the chilomonads live indefinitely.

But what are the effects of exposure to low temperature on the frequency of division at temperatures in which the chilomonads do not live indefinitely? The following experiment answers this question.

Chilomonads were transferred from the "stock cultures" used in the preceding experiments to temperatures of 35° and 30° C. The chilomonads always die eventually at 35° independent of their treatment; at 30°, however, they normally live and flourish indefinitely. The results are presented in Fig. 3.

In the figure, the "percent surviving" was obtained by dividing the number of organisms living at the end of an observation period by the number introduced at the beginning and multiplying the result by 100.

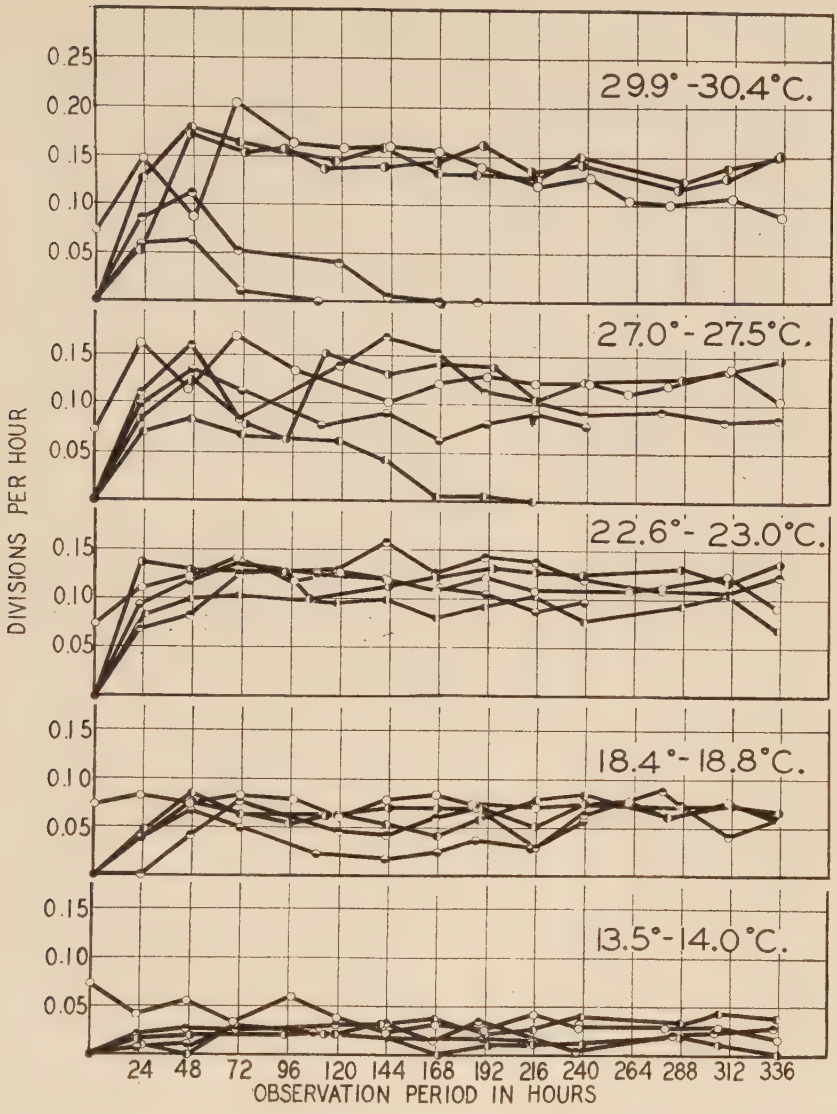

FIG. 2. The effects of prolonged exposures to low temperatures on the frequency of division at temperatures in which chilomonads live indefinitely.

○, exposed to 13.5° C. for 1 week.
◑, exposed to 13.5° C. for 1 week, then to 9.5° C. for 1 week.
◐, exposed to 13.5° C. for 1 week, then to 9.5° C. for 2 weeks.
◓, exposed to 13.5° C. for 1 week, then to 9.5° C. for 3 weeks.
◒, exposed to 13.5° C. for 1 week, then to 9.5° C. for 4 weeks.

Obviously, this "percent surviving" is the result of two opposing factors: the reproduction of the organisms on the one hand, and death of the organisms on the other hand.

Figure 3 shows that after the same period at 35° C., the percentage surviving decreased as the exposure to 9.5° C. increased. The figure also shows that after the various exposures to 9.5° C., the percentage surviving decreased as the exposure to 35° C. progressed. Furthermore, the figure shows that as the exposure to 9.5° C. increased, the time chilomonads lived at 35° C. decreased.

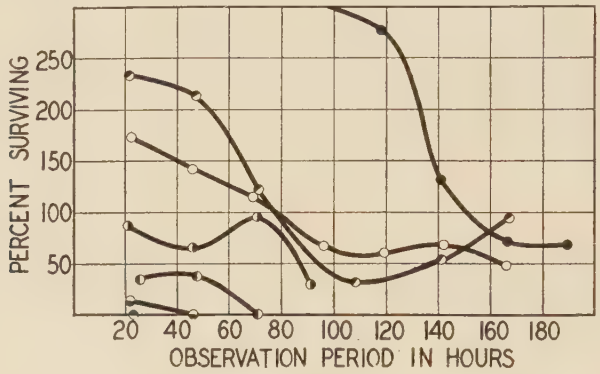

FIG. 3. The effects of prolonged exposures to low temperatures on the frequency of division at temperatures in which chilomonads do not live indefinitely.

○, exposed to 13.5° C. for 1 week, then observed at 35° C.

◑, exposed to 13.5° C. for 1 week, then to 9.5° C. for 1 week, then observed at 35° C.

◐, exposed to 13.5° C. for 1 week, then to 9.5° C. for 2 weeks, then observed at 35° C.

◓, exposed to 13.5° C. for 1 week, then to 9.5° C. for 3 weeks, then observed at 35° C.

◒, exposed to 13.5° C. for 1 week, then to 9.5° C. for 4 weeks, then observed at 35° C.

●, exposed to 13.5° C. for 1 week, then to 9.5° C. for 3 weeks, then observed at 30° C.

◐, exposed to 13.5° C. for 1 week, then to 9.5° C. for 4 weeks, then observed at 30° C.

It was noted above in Fig. 2 that the temperature of 30° C. acted as a viable temperature except when the chilomonads had been exposed to 9.5° C. for 3 and 4 weeks, respectively. Figure 3 also illustrates the effects of this low temperature on the percentage surviving at 30° C.: the same three phenomena evident at 35° C. appear. Experimental error accounts for the final rise in the percentage surviving of chilomonads subjected to 9.5° C. for 4 weeks.

Several conclusions may be drawn from these experiments. First,

exposure to low temperatures modifies the organisms in such a way that the frequency of division is low for a short time after transfer to higher temperatures. Second, the same modification apparently lessens the vitality of the organisms, because the ability to withstand detrimental effects of lethal high temperatures is reduced. Third, at temperatures in which the chilomonads live indefinitely the effects of the exposure to low temperature do not persist longer than 48 hours, but in other temperatures the organisms exhibit a decrease in survival that is in direct ratio with the length of exposure to low temperature.

Certainly the exposure to low temperature does modify the chilomonads. Modifications of this sort have been noted before. Dallinger (1887) induced modifications in certain monads that enabled them to live at very high temperatures and made it impossible for them to survive at the temperature in which unmodified monads flourished. Hance (1915) notes that a particular strain of *Paramecium caudatum* was able to resist higher temperatures than most and says that the former might have arisen in experiments dealing with high temperatures. Thus the present experiments serve to verify other observations, showing that organisms can be modified by environment and that the modification can persist for some time. The nature of the modification will be discussed again below under the effects of temperature on the changes in the volume of starch and fat.

The Effect of Temperature on the Volume of Starch and Fat in Chilomonas paramecium

The body of *Chilomonas paramecium* under conditions of optimum nutrition and temperature contains a fairly uniform number of particles; some of these give reactions characteristic of starch, some characteristic of fat. Under adverse conditions, the number and size of the particles may decrease or increase markedly. Mast and Pace (1932*a*, 1932*b*, 1933, 1935, 1937, 1938) make observations on the starch and fat of *Chilomonas* in solutions containing various substances always at one constant temperature. In their studies, they examined several chilomonads, then made a camera lucida drawing of a typical organism or of one taken at random. The present experiments were undertaken, first, to measure the actual volume of starch and fat in chilomonads, second, to observe the effects of different temperatures on the volume of starch and fat, and third, to ascertain the relation between the starch and fat and the frequency of division.

1. *In acetate ammonium solution.*—Experiments to ascertain the effects of various temperatures on the starch and fat under optimum conditions of nutrition were performed to supplement those of Mast

and Pace on starch and fat under various conditions at one constant temperature.

The organisms, solutions, chemicals, and glassware were precisely the same as in preceding experiments. The same methods were used to maintain sterility of the solutions, and the results appearing in this paper were obtained with solutions sterile except for the presence of the experimental chilomonads.

It was necessary to have a large number of organisms for each observation. To accomplish this several were transferred instead of the single chilomonad at establishment of each new culture.

The following procedure was used to measure the volume of starch and fat in *Chilomonas.* Several chilomonads from an experimental culture were drawn into a capillary pipette with the smallest volume of fluid; these were ejected on an ordinary glass slide, and surrounded by a ring of vaseline. Then one small drop of Lugol's solution and one of a saturated solution of Sudan III in absolute alcohol were added in succession with glass rods or wire loops. A cover-glass was placed over the drop and maneuvered to hold the drop in the center of the ring. This was observed with a microscope equipped with an ocular micrometer, and the size of each particle of starch and fat recorded. Five chilomonads were measured in each observation. Statistical methods were employed to ascertain significant differences.

Preliminary experiments showed that three temperature ranges could be distinguished: (*a*) the range between about 10°–13° C. and 30° C. in which the chilomonads live indefinitely (viable temperatures), (*b*) the range above the one in which the chilomonads live indefinitely (lethal high temperatures), and (*c*) the range below the one in which chilomonads live indefinitely (lethal low temperatures). Experiments were designed to show the effects of these three ranges on the volume of starch and fat.

a. Viable Temperatures.—The results of the experiments at viable temperatures are shown in the second and fourth columns of Table II. It is there shown that the volume of starch and fat per chilomonad at all viable temperatures is the same.

In order to obtain a more nearly complete analysis of the effects of viable temperature, the total volume of starch and fat synthesized by the progeny of one chilomonad in a 24-hour period was computed. This was done as follows. The number of divisions per 24 hours and thence the number of progeny produced from one chilomonad in that period were deduced from Fig. 1. By multiplying this number by the volume of starch and fat per chilomonad, the total volume of starch and fat synthesized in this period were obtained. The results are presented in

the third and fifth columns of Table II. It is there shown that as the temperature rises within the viable temperature range, the volume of starch and fat synthesized increases.

The remarkable part of these experiments is that although the rate of synthesis of starch and fat increases as the temperature rises within the viable temperature range, the volume of starch and fat *per individual* remains the same. One of two hypotheses may explain the situation. First, this may mean that under optimum conditions of salt concentration and nutrition in viable temperatures, the rate of synthesis of starch and fat controls the frequency of division. Second, the starch and fat may be by-products of metabolism, by-products which can be utilized as food materials under adverse conditions of nutrition, and their production and the frequency of division may take place at such rates that the volume of starch and fat remains constant.

TABLE II

The effect of viable temperatures on the volume of starch and fat per individual chilomonad and on the volume of starch and fat synthesized by the progeny of one chilomonad in a 24-hour period.

Temperature	Volume of starch per individual	Estimated volume of starch produced by progeny of one chilomonad in 24 hours	Volume of fat per individual	Estimated volume of fat produced by progeny of one chilomonad in 24 hours
30° C.	299.11±85.18	3806.87	52.07±20.12	701.67
27.3°	298.27±99.68	2907.53	66.92±22.48	652.08
23.2°	297.96±88.76	1348.15	65.99±32.76	298.43
18.5°	288.13±72.54	785.62	96.56±49.92	262.02
13.5°	314.18±61.15	518.86	119.50±34.01	197.34

b. Lethal High Temperatures.—The experiments were performed like those preceding, except that temperatures above the viable temperature range were used, that transfers were made at 24-hour intervals only when the experiment lasted several days, and that observations were made at 12-hour intervals. The results are presented in Fig. 4.

The figure shows that as the temperature rises above 32.5° C. the chilomonads lived for decreasing periods of time, and the volume of starch and fat increased as the exposure to the high temperature proceeded. This increase is due to two factors. First, the rate of synthesis either increased or was uninhibited. Second, there was a direct ratio between the length of life and the amount of accumulation. These two factors acted together to cause the greatest accumulation at 35° C.

It will be recalled that in experiments cited above, the frequency of division decreased as the temperature rose above the optimum; this, as

was pointed out, was largely due to the increase in the death rate and probably also to an actual decrease in the division. The present experiment demonstrates that the synthesis of starch and fat continues above 30° C., a temperature optimum for division, since starch and fat that would go into two or more dividing organisms now accumulate in one.

Thus the synthesis of starch and fat cannot control the frequency of division at lethal high temperatures. It is obvious that the frequency of division is inhibited whereas synthesis continues.

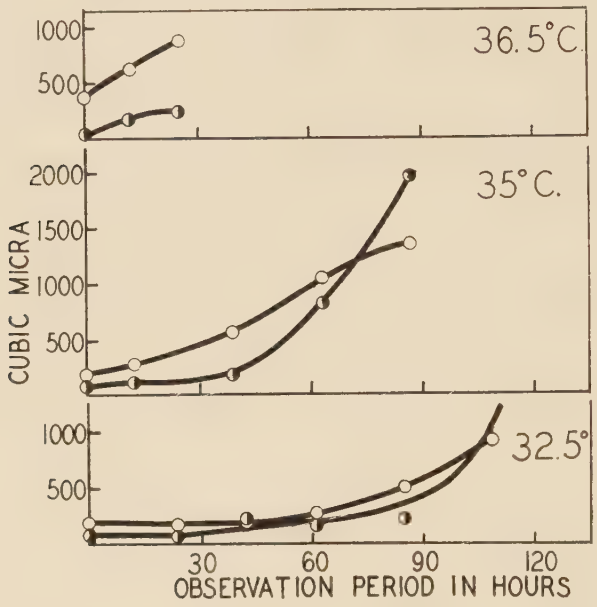

FIG. 4. The effect of three lethal high temperatures on the volume of starch and fat in *Chilomonas paramecium*.

○, volume of starch per chilomonad; ◑, volume of fat per chilomonad.

c. Lethal Low Temperatures.—These experiments were performed exactly as the preceding except as follows. A temperature of 9.5° C. was used since the organisms live for a considerable time but eventually die. Acetate ammonium solution was put into about 80 depressions, several chilomonads from a clone introduced to each, and the dishes put into the compartment at 27° C. for 24 hours until the chilomonads had become numerous. Then they were put into the compartment at 9.5° C. and observed periodically for 600 hours until the last chilomonad died. During this time the solution was replenished occasionally with fresh acetate ammonium solution. The results of this experiment are presented in Table III.

The table shows that as the period at 9.5° C. is prolonged, a decrease in the volume of starch is followed by an increase in the volume of fat. However, a statistical analysis shows that these changes are insignificant. The tendency for starch to decrease and fat to increase is, no doubt, present but is obscured by the great variation in the starch and fat in individuals under identical conditions.

It is quite probable that the modification of the frequency of division and the ability to survive at lethal high temperatures noted above as the result of prolonged exposure to low temperature is due to changes in the volume of starch and fat. However, experiments to be cited below indicate that the rate of resynthesis of starch and fat is so fast that the

TABLE III

The effect of a lethal low temperature, 9.6° C., on the volume of starch and fat per chilomonad.

Time at 9.6° C.	Volume of starch, cubic micra	Volume of fat, cubic micra
30 hours	165.4±35.9	35.9± 9.4
53	220.0±53.0	23.4± 14.0
90	138.8± 7.8	28.1± 3.1
124	37.4±25.0	57.7± 27.3
161	65.5±35.9	48.4± 39.0
211	28.1±25.0	129.5± 93.6
235	68.6±29.6	42.1± 18.7
292	51.5±10.9	84.2± 42.1
329	59.3±59.1	73.3± 25.0
353	106.1±26.5	73.3± 25.0
405	76.4±49.9	76.4± 31.2
497	71.8±54.6	173.2±138.8
521	99.8±64.0	248.0±126.4
598	64.0±28.3	224.6±163.8

modification can be accounted for only in part by changes in the starch and fat induced by low temperatures.

Since there is no division at this temperature and no increase in the volumes of starch and fat in the first 400 hours, there is no synthesis of these substances. Death could well be due to starvation in that no available food products are synthesized.

The decrease in the volume of starch followed by an increase in fat indicates that starch is probably transformed into fat.

2. *In Acetate-free Solution.*—Mast and Pace (1932*a*, 1932*b*, 1933) showed that if sodium acetate is omitted from solution, the volumes of starch and fat decrease to zero, and the chilomonads die of starvation. Their experiments were performed at one constant temperature, and they

made no actual numerical measurements of the volumes of starch and of fat. The present experiments were designed to ascertain the effects of temperature on the process of starvation of chilomonads in acetate-free solution and the effects of temperature on the recovery from starvation in acetate ammonium solution.

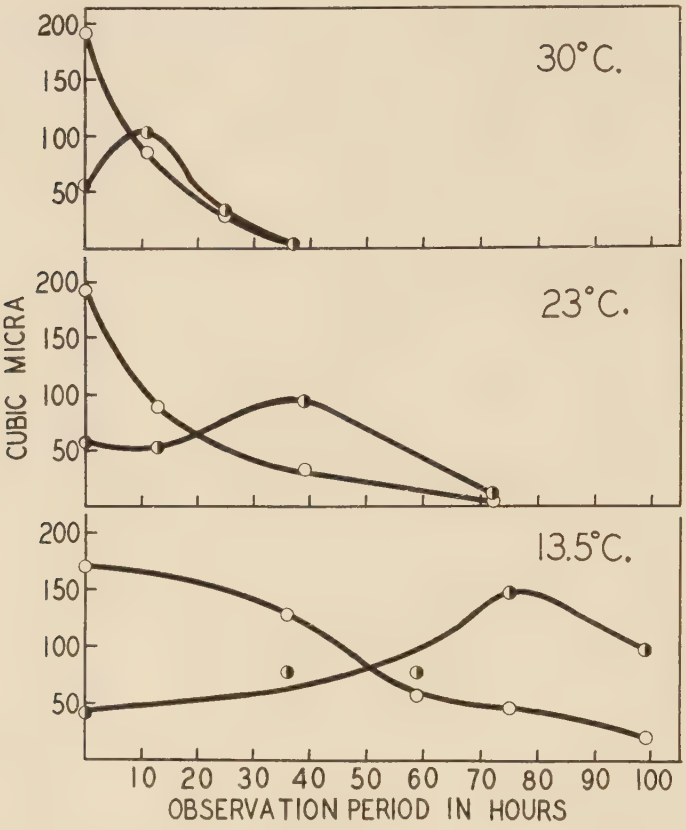

FIG. 5. The effect of viable temperatures on the change in the volume of starch and fat of chilomonads in acetate-free solution.

○, volume of starch per chilomonad; ◑, volume of fat per chilomonad.

Glassware and pipettes were the same as in the preceding experiments. The acetate-free solution described in Table I was used as the culture medium. The hydrogen ion concentration was adjusted to pH 6.8 with hydrochloric acid or potassium hydroxide, both prepared from boiled redistilled water. The Petri dishes were prepared as in the preceding experiments, except that a saturated solution of barium hydroxide

was poured into the bottom; this served to decrease the concentration of carbon dioxide in the atmosphere of the dish and to prevent the condensation of water beneath the depression slides.

A large number of chilomonads was transferred through four depressions containing acetate-free solution and then to the experimental acetate-free solution. It was then put into the constant temperature bath. No further transfers were made. The results of this experiment are presented in Fig. 5.

Figure 5 shows that the volume of starch decreased to a minimum, that the volume of fat first increased, then decreased to a minimum, and that these changes in the volumes of starch and of fat took place faster at higher than at lower temperatures.

Thus the same reaction occurs at the three temperatures studied; temperature acts as an agent that influenced the speed but not the processes involved in the reaction.

The changes noted here are similar to those indicated by the experiments cited above on the effects of lethal low temperatures. From these two sets of experiments, one can assume that under conditions in which there is no synthesis, the starch is gradually consumed, perhaps partially transformed into fat, and then the fat is consumed.

A second group of experiments was performed similar to the preceding, except that starved chilomonads obtained from cultures prepared as in the preceding experiment, were transferred directly to acetate ammonium solution. Synthesis of both starch and fat was rapid, requiring about 12 hours at 30°, about 15 hours at 23°, and about 18 hours at 13.5° C. for the volumes to increase from zero to the maximum typical of chilomonads grown in viable temperatures.

Discussion

The use of change in the volume of starch and fat as indicative of changes in metabolism of chilomonads raises three questions. First, is the method described in this paper sufficiently accurate to measure any changes in the volume of starch and fat? Second, is the change in the volume of starch and fat actually indicative of changed metabolism? Third, is the volume indicative of the mass of starch and fat?

The method is seldom very accurate. This is due to three factors: first, measurement of all particles is optically difficult; second, the particles are not always spherical, and their spherical diameter must be estimated; third, there is considerable variation in organisms kept under identical conditions. However, the method is undoubtedly more accurate than comparing organisms by means of camera lucida drawings or esti-

mating the relative amounts of starch and fat from a series of such drawings.

It is the author's contention that the volume of starch and fat may be indicative of the metabolic rate of the organism. The entire body of chilomonads living in acetate ammonium solutions, in natural ponds, or in rice cultures, is always well filled with starch and fat. This condition probably results from a balance between synthesis and decomposition of starch and fat; it is likely that in such an environment the chilomonads have a characteristic metabolic rate. A modification of the environment might upset this balance, causing a change in the rate of synthesis or decomposition, and subsequently, a change in the volume of starch and fat. Such changes would be accompanied by an alteration of the metabolic rate to a new and different characteristic value. Changes in the volume of starch and fat occurred and were described above; these were probably accompanied by changes in the metabolic rate.

The volume has been assumed in this paper to be an index of the mass of starch and fat. This is true if there are no changes in the chemical composition which alter the specific gravity of the starch and fat. Temperature is known to alter the iodine number of fats extracted from living organisms, but no experiments have been done on chilomonads to ascertain changes in the chemical composition of their protoplasm.

Summary

1. *Chilomonas paramecium* was grown in a solution of inorganic salts plus sodium acetate, and the effect of temperature on the frequency of division and on the volume of starch and fat measured.

2. As the temperature rises the frequency of division increases from zero at 9.5° C. to a maximum at 27°–30° C. and then decreases to zero.

3. When chilomonads are transferred from low temperatures to higher temperatures, a period of about 48 hours is required before the frequency of division increases to the value characteristic of the higher temperature.

4. When chilomonads are exposed to the low temperature of 9.5° C. for prolonged periods, then transferred to higher temperatures, the lethal high temperature and the period required to kill the organisms decrease. This indicates that the low temperature modifies the organisms and that the modified condition persists for some time.

5. At all viable temperatures, the volume of starch and fat per chilomonad remains constant, but as the temperature rises from 9.5° C., the volume of starch and fat synthesized increases, reaching a maximum at 30° C.

6. When organisms are exposed to lethal high temperatures there is an increase in the volume of both starch and fat. This is undoubtedly due to the fact that synthesis at these temperatures is not greatly impeded, that division decreases or ceases, but that the organisms live for a time and accumulate some starch and fat.

7. At lethal low temperatures, division ceases, there is a gradual decrease in the volume of starch accompanied by an insignificant increase in the volume of fat.

8. In solutions without sodium acetate, there is a decrease in the volume of starch to zero, an initial increase and a subsequent decrease in the volume of fat to zero. This process is identical at all viable temperatures but proceeds faster at the higher temperatures.

9. Synthesis of starch and fat by starved chilomonads in acetate ammonium solution is rapid and varies directly with the temperature.

10. The method of observing and measuring the volume of starch and fat has several drawbacks but does indicate changes with some accuracy.

11. The volume of starch and fat may indicate the metabolic rate of the organisms. Chilomonads living in an environment optimum for nutrition and temperature are filled with starch and fat and probably have a characteristic metabolic rate resulting from a balance in the rates of synthesis and decomposition of starch and fat. Changes in the environment may result in the breakdown of the balance, change in the volume of starch and fat, and probably a change in the metabolic rate.

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